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Phenology and Bionomics of *Culicoides* (Diptera: Ceratopogonidae) in Glynn County, Georgia

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submitted by

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ABSTRACT

Seasonal abundance and population densities of *Culicoides* species from sites in Glynn County, Georgia were surveyed using 15 New Jersey light traps during a 9-month period from April-December 1990. Environmental effects on the abundances were also analyzed. The three primary species present were: *Culicoides furens* (Poey), *C. hollensis* Mellander & Brues, and *C. melleus* (Coquillett). *C. furens* were collected year round, but predominantly during the summer. *C. hollensis* had a bimodal abundance (spring and fall). *C. melleus* also had a bimodal abundance (late summer and fall).

Larval habitats of the primary species were identified from the salt marshes adjacent to two different sites, Sea Island and St. Simons Island. Soil samples were taken from the sites at least monthly from February 1990 through April 1991. Three zones primarily differentiated by elevation, were identified at both of the sites. Most (89%) of the total larvae of all three species combined were found within Zone II caracterized by intermediate (0.3-1.5 m) *S. alterniflora* Loiseleur and 11% of the total larvae were collected from Zone III characterized by *Juncus roemerianus* Scheele, *Iva frutescens* Linnaeus, and *Spartina patens*

(Aiton) Muhl. At Sea Island, 78% of the larvae were collected from Zone II, whereas 22% were collected from Zone III. On St. Simons Island, 97% were collected from Zone II, whereas only 3% were collected from Zone III. No larvae were collected from Zone I at either site characterized by tall *Spartina alterniflora* (>1.5 m in height).

Soil samples were collected from a salt marsh on the north end of St. Simons Island. Thermal preferences for 3rd and 4th instar larvae of two field collected coastal species, *C. furens* and *C. hollensis* were determined. Larvae were placed in an estuarine-water filled (salinity 2.8 g/dl, pH 5.5) stainless steel trough with one end resting on an ice pack (22.6°C) and the other resting on a hot plate (42.8°C). Temperature preferendium for *C. furens* was 30.0-39.9°C (57%, $P \leq 0.05$), whereas *C. hollensis* preferred 20.0-29.9°C (72%, $P \leq 0.05$).

Two organophosphates (dibrom and temephos) and two pyrethroids (permethrin and resmethrin) were tested in the laboratory for efficacy of control for biting midge larvae. Field collected larvae from a salt marsh on north St. Simons Island were exposed to four larvicides in either water alone or water with soil substrate. Temephos showed optimal control against biting midge larvae, while having low toxicity on the non-target organisms (other arthropods) tested.

INTRODUCTION

Culicoides spp., biting midges in the family Ceratopogonidae, are known by many names in various places: "sand gnats" in coastal Georgia, "sandflies" or "no-see-ums" on the Atlantic and Gulf coasts of the United States, "moose-flies" in Alaska, "no-no's" in Polynesia, and "jejenes" in Spanish-speaking Latin America (Wirth & Hubert 1989). Biting midges are pests of man and livestock in the coastal zone along the Atlantic Seaboard. *Culicoides* spp. are known to transmit a number of viral diseases to man and animals, diseases including Rift Valley Fever in man, Bluetongue disease in cattle and sheep, and Epizootic Hemorrhagic Disease in deer. In addition to viral diseases, *Culicoides* spp. also transmit a number of protozoan and filarial parasites (Linley et al. 1983). Their bites can cause red wheals, and in extreme cases can cause cellulitis and other severe allergic reactions. Aside from their medical and veterinary importance, these insects have a significant impact on outdoor recreation and tourism in the coastal zone.

To date there have been few studies done on the biology of biting midges in Georgia. Most earlier studies focused on the noncoastal species (Foote & Pratt 1954, Williams 1955, Ah 1968). However, a recent study surveyed the seasonal abundance of adult coastal species in Georgia

(Magnon & Hagan 1988).

Studies have been made on the larval habitat of salt marsh *Culicoides* spp., for example the work of Carpenter (1951) in the Panama Canal Zone, Bidlingmayer (1957) in Vero Beach, Florida, and Kline & Wood (1989) in Yankeetown, Florida, but the only study to date on the larval habitat of biting midges in coastal Georgia was by Magnon et al. (1990).

Numerous studies have been done on larval ecology and biology (Linley 1966, Kettle 1969, Davis 1981). *Culicoides* larvae have been found in a variety of habitats, including salt marshes, tree holes, ponds, and sandy intertidal zones. Larval development requires an optimum temperature and it is known that the rate of development is inversely related to temperature (Davis 1981). Thus, finding the temperature preferendium would provide yet another key in the biology of larvae.

Linley and Adams (1972) studied the preferred temperature range of *C. melleus*, but no study has been made for the thermal preferences of the two other primary pest species. Understanding more of the ecology and biology of larval *Culicoides* may aid in developing an integrated control program in the future. The present study focused on the two other species, *C. furens* and *C. hollensis*.

Along the Georgia coast three primary species of biting

midges, *Culicoides furens*, *C. hollensis*, and *C. melleus* are nuisances to both man and animals. Controlling adult biting midges may be accomplished by using aerial spray applications or ground applications. Most control measures for adults to date have been unsuccessful due to the flight range and movements from breeding sites (Breeland & Smith 1962). However, control by adulticiding provides only temporary relief. Therefore, larval control would seem to be a more effective way to reduce the numbers of adult *Culicoides*, and provide more long term reduction in populations.

Numerous studies have been conducted on toxicity and effectiveness of various insecticides on larval *Culicoides*. Early studies for control of biting midges were made on the effectiveness of chlorinated hydrocarbon pesticides e.g. DDT, lindane, and dieldrin, which were sometimes called "first generation pesticides." (Jamnback et al. 1958, Wall & Doane 1965, Fox et al. 1968). These insecticides were effective, however, the long term residual effects were found to be environmentally unsound and their use is no longer approved. Therefore research by industry was begun to develop other insecticides.

In field tests against *Culicoides* larvae in Massachusetts, Wall and Marganian (1971 & 1973) reported success in controlling larvae with the "second generation

pesticides", organophosphate and carbamate pesticides, temephos, malathion, and chlorpyrifos with little adverse effects on other intertidal fauna. Temephos seemed to be a promising agent in controlling biting midge larvae (Kay et al. 1973, Campbell & Denno 1976, Ferguson & Cawthorne 1980).

Major objectives of the present study were to compare population densities and seasonal distribution of adult *Culicoides* spp. in Glynn County and test for correlation with environmental effects (i.e. moon phase and tidal heights) on abundances along with determining densities of larval *Culicoides* spp. Temperature preferendia for two of the pest species will be examined. Lastly, efficacy of control of four insecticides will be evaluated. Upon the conclusion of these studies of the biology and ecology of adult and larval *Culicoides* spp. along the coast of Georgia, it is hoped that an integrated control program may be developed to reduce populations below the nuisance threshold.

METHODS & MATERIALS

Study Sites

The research area was located in Glynn County, Georgia in the lower Atlantic Coastal Plain region (Fig. 1). The county occupies 1,272 km² and is bound on the south and east by approximately 34 km of irregular Atlantic Ocean shoreline. Elevations range from sea level to 15 m (Mathews et al. 1980). Average yearly rainfall ranged from 4.97 cm to 19.1 cm with mean annual rainfall of 10.7 cm from 1951-1975 in Brunswick. Temperatures ranged from 11.1 to 27.8°C with mean annual temperature of 19.4°C from 1951-1975 (Rigdon & Green 1980).

The county is drained by three significant river systems: Altamaha, Turtle-Brunswick, and Little Satilla. The Turtle-Brunswick and Little Satilla Rivers, which form a portion of the southern boundary of the county, are coastal rivers with flow dominated by tidal action with salinities ranging from 5 to 30 PPT. The Altamaha River which forms the northern boundary of the county, is dominated by freshwater discharge the entire year (Mathews et al. 1980).

Sea Island in Glynn County, Georgia (81°20'W, 30°12'N) is a Holocene barrier island 7.9 km long and 3.0 km wide with maritime forest communities located on beach ridges

(Magnon & Hagan 1988). Elevations range from sea level to 6.7 m above sea level (Mathews et al. 1980). The larval research site has previously been described by Magnon et al. (1990) (Fig. 2). According to D.V. Hagan (unpublished data), soil types from this area consisted of the following: in the higher elevations, the soil consisted of sand; the intermediate areas consisted of a thin layer of sand covering mostly clay; in the low lying areas, the soil consisted of sand and silt.

St. Simons Island, also in Glynn County (81°20'N, 31°17'W), is a Pleistocene sea island with Holocene beach ridges. The island is 17.7 km long and 4.8 km wide with elevations ranging from sea level to 7.6 km at the top of the natural beach ridges (Mathews et al. 1980). The larval research site was a marsh covered predominantly with smooth cordgrass *Spartina alterniflora* Loiseleur ranging from 0.3 to 1.5 m in height. The marsh soil was saturated with water even at low tide. Vegetation was dense and the soil appeared matted due to the dense plant root system. The area highest in elevation consisted of sand (75%) and clay (25%), and the lowest area was composed of clay (95-100%) (Westberry & Hagan unpublished data).

Three zones were described for St. Simons Island (Fig. 3). Zone I was characterized by tall *Spartina alterniflora* (>1.5 m in height). The largest area of the marsh was Zone

II, this zone was characterized by intermediate (0.3-1.5 m) *S. alterniflora* only. Zone III was the highest area which separated the marsh from the maritime forest. This zone differed from Zone III at Sea Island by the presence of *Spartina patens* (Aiton) Muhl, *Juncus roemerianus* Scheele, and *Iva frutescens* Linnaeus.

Population Density

The 15 New Jersey light traps were placed at specific locations in Glynn County (Fig. 4). The light traps were operated and maintained by the Glynn County Mosquito Control Commission, twice a week (Tuesdays and Thursdays) April through December 1990. Light traps were not operated by the Glynn County Mosquito Control Commission during the summer months and were only operated one month during the winter. The insects were removed from the traps and placed in containers marked with light trap number and date collected. The technicians at the Glynn County Mosquito Control Commission removed the mosquitoes and then provided the smaller insects for this study. Collections were later transported to Georgia Southern University for sorting and counting.

In the laboratory, containers were emptied into a petri dish and examined with a Nikon dissecting microscope at 80X, equipped with a Bausch & Lomb Fiber-Lite. Numbers of each

species were recorded and results tabulated.

Collection and Extraction of Larvae

Two methods were used to collect soil samples for this study. For finding temperature preferendia and evaluating larvicides, approximately 50 samples were collected from the top 5 cm of the surface using a hand trowel. To study larval distribution, an average of 50 samples of soil was collected using post-hole diggers along a transect (60-130 m). In both methods, about 1200 ml of soil was placed into each container.

Field collected soil samples were protected from temperature extremes by removing from direct sunlight after collecting to reduce larval mortality and then were brought to the laboratory at Georgia Southern University. After 24 to 36 hours, larvae were extracted using the agar method of Kline et al. (1981). The soil in the collection containers was leveled and 300 ml of melted agar (cooled to 47°C) was poured on the top of the soil. After the agar hardened, tap water was poured over the agar to prevent the agar from drying. After 20-24 hours, the water was poured into a black photographic developing tray. Larvae were located, removed with a pipette and placed into a separate container.

Larval Distribution

Soil samples were collected from Sea Island from February to September 1990 and in January 1991. Samples were collected from St. Simons Island from October to December 1990 and then again from February to April 1991. The 13 plots sampled from Sea Island yielded approximately 65 samples. Five samples were taken every 2 m in each 10 X 10 m plot. The soil from St. Simons Island was collected in the same manner, except that only approximately 30 samples were collected. In the laboratory the soil samples were processed as above using the agar extraction method and the number of larvae recovered per plot was recorded.

Temperature Preferendia

To determine the preferred temperatures for larval *Culicoides* species, the following apparatus was designed (Fig. 5). A stainless steel pan (29.0 X 18.4 X 12.9 cm) was placed on two temperature extremes. Estuarine water (500 ml, 1.2 cm in depth) was placed in the pan. The cold extreme (22.6°C) was produced by placing two ice packs under one end. The hot extreme (42.8°C) was produced by placing the pan on a hot plate regulated by a rheostat.

Field collected estuarine water (salinity 2.8 g/dl, pH 5.5) from the Hampton River off north Sea Island was held in the laboratory at room temperature before being placed in

the pan. Larvae were then placed into the center of the pan and allowed to swim freely for ≥10 min. The pan was placed under uniform fluorescent lighting, to insure the entire area of the pan was equally illuminated. Shading of areas was avoided, since larvae are known to be negatively phototactic (Davis 1981). Ten larvae of each species were used for each trial. Three trials were made with three replications per trial for a total of nine replications.

Larvae were allowed to acclimate in the pan for ≥10 min, before the readings were taken. During the experiment, larvae were removed with a pipette and the temperature read with a digital thermometer (Reotemp Thermistor, CR-1) at the precise location from which each larva was removed.

Evaluation of Insecticides

To find the appropriate concentrations for each larvicide, 25 ml of estuarine water was placed in 266 ml Solo plastic cups. Half the cups contained only water and the other half contained both substrate and water. Those cups with substrate contained 5 ml of substrate from areas of known larval habitat and 25 ml of estuarine water. The substrate was strained through cheesecloth to remove large pieces of sediment and debris. Stock solutions of temephos, resmethrin, permethrin, and dibrom were diluted to 1,000, 4,000, 7,000 and 10,000 PPB.

One milliliter of each test dilution was placed in the cups to allow the agent to combine with the water and water plus substrate, before larvae were added. Duplicates were made of each dilution. After larvae were extracted from the marsh soil, they were placed into 24-well tissue culture plates which provided easier access to the larvae. Approximately, four 3rd-4th instar larvae were added to each cup. Larvae were identified to species after each trial from a composite key constructed by Magnon et al. (1990) based on descriptions by Wirth (1952), Jamnback et al. (1958), and Linley & Kettle (1964). The controls consisted of active ingredient dissolved in acetone which was later diluted using water.

To test other arthropod non-target organisms (e.g. *Uca* spp. and *Sesarma cinereum*), the same procedure was followed except that only one test was performed. Tests were performed with either water alone or substrate plus water. Because of their aggressive habits only one fiddler crab, *Uca* spp. was placed in each cup. Controls were maintained also, with water alone and substrate plus water.

Containers for testing efficacy of various dosages of pesticides were held in the laboratory at two temperatures, 19±1°C or 21±1°C for 96 hours, after which mortality readings were taken. Larvae were considered dead if they did not respond with the usual serpentine-like motion when

touched with a probe.

Statistics

Data were statistically tested for trends between adult biting midge abundance and various environmental factors. Moon phase and abundance were analyzed using a Mann-Whitney U, whereas, tidal magnitude and abundance of adult biting midges were tested using multiple regression (Statview 512+ 1986). Data from all larvicide efficacy tests were summed and the dose-response relationship determined by probit analyses of the log-transformed mortality data (Finney 1971) using the SAS PROBIT procedure (Ray 1982).

RESULTS AND DISCUSSION

Seasonal Abundance and Population Density

A total of 7,013 adults was collected from April to December 1990. Of the total collected 70.7% were *C. furens*, 27.1% were *C. hollensis*, and the other 2.2% were *C. melleus*. *C. furens* was collected year round, but predominantly during the summer months (May and August). Peak months for *C. hollensis* were April and October. *C. melleus* was collected mainly in the late summer and fall months (Table 1).

Light trap collections were analyzed for peak abundances for each species from April through December 1990. Light traps (LT) with the highest collections were numbers 1, 2, 4, 9, 10 and 14, with LT 1 having the highest collections in the county. Of the 7,013 collected, 1,921 (27%) were collected at LT 1 (Table 2). LT 9 was the next highest in collections (19%). The three light traps with the highest density were examined more closely to ascertain any trends. *C. furens* were most abundant at LT 1, 4 and 9 (Fig. 6). *C. hollensis* were most abundant at LT 1, 2 and 9 (Fig. 7). The peaks for *C. melleus* were at LT 1, 10 and 14 (Fig. 8). County-wide, *C. furens* peaked in August, *C. hollensis* peaked in April and *C. melleus* had peak abundances in August and September.

In North Carolina, Kline & Axtell (1976) reported an abundance for C. furens in the spring (late April) through the fall (early October). Khalaf (1967) found *C. furens* occurred in high numbers late in the fall when *C. hollensis* was almost absent in Louisiana. In west Florida studies, *C. furens* was present in large numbers from May to September, and the greatest abundance occurred in May and June (Kline & Roberts 1982). In Yankeetown, Florida, *C. furens* was present only during the warmer months with the highest numbers in September (Kline 1986). Our study indicated that *C. furens* was found year round but had highest abundance in the summer.

Results of this study when compared to data collected in other regions supports the observation that the seasonal incidence of *C. furens*, *C. hollensis*, and *C. melleus* on the Atlantic coast of the United States varies with latitude. The incidence was shorter in more northern latitudes and longer in the more southern latitudes.

C. hollensis was most abundant in this study in the spring. Similarly, Beck (1952) found that in Florida, *C. hollensis* (known earlier as *C. canithorax*) was present in great numbers in the spring where she also found greater numbers in March, with smaller numbers from March to May (Beck 1958). In contrast to our study, in North Carolina, *C. hollensis* first appear and peaks in early April (Kline &

Axtell 1976). Magnon & Hagan (1988) found *C. hollensis* to be most abundant from March through April in Glynn County.

In Glynn County, *C. melleus* was most abundant in the late summer and fall. *C. melleus* was restricted to coastal zones and rarely occurred inland. The collections in Louisiana only found this species more abundant between early April and mid-June (Khalaf 1969), this corresponds with data recorded by Beck (1958) in Florida where this species was most abundant in March through May. Kline (1986) found that *C. melleus* in North Carolina was present from March through October with peaks from April through June. He also found that *C. melleus* adults generally were present from early April to about mid-October.

One explanation for the low number collected could be that *C. melleus* was not attracted to the light in the traps (Kline 1975, Magnon & Hagan 1988). However, Beck (1952, 1958) and Khalaf (1969) used New Jersey light traps. Another explanation for the low numbers could be that *C. melleus* is not as abundant on the Georgia coast as in other areas. Magnon & Hagan (1988) found that *C. melleus* comprised only 11% of the total number collected. Therefore, one can assume that light traps are not effective in determining abundances of *C. melleus*.

Environmental Effects

In comparing environmental effects on the totals of each species, the light trap with the highest abundance overall (LT 1) was used to determine correlations between adult collections and moon phase. No observable relationship was found between the phase of the moon and the abundance of adult *Culicoides* spp. (Table 3). Also no relationship was observed between tidal magnitude and adult biting midge abundance.

Several studies have been conducted on the effect of moonlight on flight activity of *Culicoides* and other Diptera such as mosquitoes (Williams et al. 1956, Provost 1959, Bidlingmayer 1964, 1967, 1971, Bowden 1973). My results indicated no relationship between the number collected and the phase of the moon. However, one possible explanation could be an insufficient data set size. While no significant relationship was observed, my data visually showed a trend for a relationship. The data showed *C. furens* was collected in greater abundances around full moon and *C. hollensis* around new moon. No trends were apparent for moon phase and *C. melleus*. As stated previously, *C. melleus* may not to be attracted to light; if so, then no pattern might would be expected.

Bidlingmayer (1964) found no significant differences in the numbers of mosquitoes captured by light traps between a

full moon and new moon. However, he found a 546% increase in light trap collections on full moon. *C. furens* in Glynn County increased in August by 37.3% on full moon over new moon and in October a 1141% increase over a moonless night. *C. furens* in this study had two peak abundances on new moon and full moon. The nights before full moon yielded the highest collections. From preliminary studies on biting/landing rates, *C. furens* has been found to be crepuscular in its temporal distribution (D.V. Hagan, personal observation). Bidlingmayer (1964) found that light intensity of the full moon was similar to the light at dawn and dusk, during which time increased flight activity occurred. The night of new moon yielded the next highest collections.

Provost (1959) in his studies found greater catches of mosquitoes at new moon rather than full moon. The reduced capture near full moon was generally thought to be caused by competition of the light trap with the moonlight. Williams (1971) also found that the presence of trees and shrubs could have reduced the number collected. Williams et al. (1956) found no lunar cycle in his light trap collections of biting midges. In the present study, *C. melleus* showed no lunar cycle in the number collected. At the first quarter of the moon phases and last quarter significant numbers of *C. melleus* were collected.

Larval Distribution

A total of 631 larvae was collected from both sites in monthly transects, 268 larvae from Sea Island and 363 larvae from St. Simons Island (Fig. 9).

In Zone II, 560 larvae, 89%, (208 from Sea Island and 352 from St. Simons Island) was collected. This zone was characterized by intermediate *Spartina alterniflora* (0.3-1.2 m). At Zone III (characterized previously) 71 larvae, 11%, were collected (60 and 11 at Sea Island and St. Simons Island, respectively). On Sea Island, 78% of the larvae were collected in Zone II, while 97% were collected from Zone II on St. Simons Island. In Zone III, 22% were collected on Sea Island and 3% were collected from St. Simons Island (Fig. 10).

The number of larvae varied with the type of soil from which the larvae were removed. On Sea Island, a majority of the larvae were recovered from a sand/silt mixture. On St. Simons Island, a sand/clay mixture yielded large numbers of larvae. In Zone I (the lowest elevation), no larvae were collected from either site.

The first study on *Culicoides* spp. larval habitat was done in what is now Belize, Central America (Painter 1926). He found *C. furens* emerging from brackish pools, edges of freshwater ponds, and from nearly pure sand which was surrounded by sea water. The vegetation in which he found

C. furens emerging was *Juncus*, sedges, and a cat-tail marsh. Other salt marsh habitats for larval *Culicoides* have been described by Hull et al. (1934) in South Carolina, Bidlingmayer (1957) at Florida, and Jamnback et al. (1958) in New York and by Hair et al. (1966) in Virginia. Salt marsh species such as *C. furens* have also been collected from other habitats, for example from fresh-water pools (Williams 1964) or sandy intertidal zones (Kline & Axtell 1975).

Results indicate that significantly more larvae were recovered from the salt marsh Zone II (-0.15 to 0.3 m). Magnon et al. (1990) recovered large numbers of larvae in a narrow Zone I adjacent to the Hampton River on Sea Island. One reason for the difference was that on St. Simons Island, the predominant marsh area was characterized as Zone II dominated by *S. alterniflora*. The plant cover was dense and provided suitable habitat for larvae. Zone II was inundated during high tide, but not flooded for long periods. However, the St. Simons Island larval research site was covered by water even at low tide. Another reason for the higher numbers in Zone II was that from October to December 1990, the soil samples were collected using hand trowels in which only the top layer of the soil was collected and thus increased the number of larvae which could be extracted.

Spartina alterniflora has been reported previously by

Kline & Axtell (1977) and Magnon et al. (1990) as suitable larval habitat for *Culicoides*. Kline (1975) found in North Carolina, that *C. furens* and *C. hollensis* were recovered from intermediate to tall *S. alterniflora*. He found the majority of the larvae were recovered from tall *S. alterniflora* habitat. Even though species of larval *Culicoides* were not identified in our survey, recent sample collections from St. Simons Island have shown that *C. hollensis* and *C. furens* were recovered exclusively.

Kline (1975) recovered *C. furens* from short *S. alterniflora* and *C. hollensis* from tall S. *alterniflora*. Wall & Doane (1960) in Massachusetts and Hair et al. (1966) in Virginia also found larvae abundant in tall *Spartina alterniflora*. In comparison with my study, no larvae were recovered from the tall *S. alterniflora* at the river's edge. Kline (1975) also hypothesized that density of root structure might influence larval distribution. Numbers of larvae recovered from St. Simons Island could be a result of this feature because the dense plant cover on top of the soil might provide suitable habitat (i.e. protection and food availability).

The collection of no larvae from these areas suggests several possible explanations. The larvae are known to be in the top 2 cm of the soil (Linley 1966), and using post-hole diggers may have decreased the amount of soil which was

collected as explained previously. Soil type was significantly different in our study than in the study by Kline (1975). The soil on St. Simons Island was a mixture of clay and sand. On the edge of a tidal creek, the soil was composed mainly of clay (95-100%). This soil may not provide suitable habitat for larvae, probably by making movement difficult for larvae.

Temperature Preferendia

C. furens had a temperature preferendium of 30.0-39.9°C with a $P \leq 0.05$ (Table 4). The mean temperature was 32.1±0.74°C (mean ± SE) with the overall range of temperatures from 22.6 to 42.8°C. The preferred temperature within the temperature range was defined as those temperatures encountered more frequently than the other temperatures. A majority (57%) of the larvae were found in the preferred range. The other 38% and 6% were found between 20.0-29.9°C and 40.0-49.9°C respectively (Fig. 11).

C. hollensis had a temperature preferendium of 20.0-29.9°C (Table 4). The mean temperature was 29.1±0.68°C with the overall range of temperatures from 23.9 to 38.0°C. Of the total, 72% of the larvae were found within the preferred range, while the other 28% were found between 30.0-39.9°C (Fig. 12).

The results of this study indicate that each of the two

species prefer different temperature ranges. In a frequency distribution, 57% of the total number of *C. furens* occurred within the preferred range. The temperature preferendium for *C. furens* was 30.0-39.9°C. Since *C. hollensis* is a cold weather species (Beck 1958, Khalaf 1967, Magnon & Hagan 1988), one might expect that larvae of this species would prefer cooler temperatures. The majority (72%) of *C. hollensis* were found between 20.0-29.9°C in the laboratory studies.

Culicoides melleus, the other of the major pest species, was not as abundant as the other two species (Magnon & Hagan 1988). In our study, *C. melleus* was not examined. Linley and Adams (1972) found that *C. melleus* preferred temperatures between 18° and 25°C.

It is known that temperature has an effect upon larval Pratt (1954) found that larvae reared in a water temperature of 10°C had the highest survival rate. This together with the observation in Florida that many larvae are found in nature during cooler months, seemed to indicate that lower water temperatures are most suitable for larval development. This supports the results of Magnon et al. (1990) who found significantly more larvae during the cooler months of the year.

Toxicity Evaluation

First Evaluation at 19°±1°C Slopes, standard errors of the slopes, and LC_{50}'s and LC_{90}'s with corresponding 95% fiducial limits for each chemical are shown (Table 5). No mortality was seen in control beakers with water and water plus substrate added.

Slope comparisons of the four chemicals showed no grouping. Dibrom was the least toxic of them all when substrate was added. The LC_{50} and LC_{90} response interval included no definite range of concentrations. Resmethrin and permethrin had similar slopes in water and water plus substrate, along with the treatment of water plus substrate in temephos. The relative potency of these four chemicals varied slightly. Temephos was the most toxic whereas dibrom was the least toxic. The addition of habitat soil did not change the order of toxicity.

Few (8 of 32) *Uca* spp. died in the test cups. There was no mortality in the controls. For the first replicate, 97% of the larvae were *C. hollensis*, while the other 3% were *C. furens*. During the four days of the test, 4% (12) *C. hollensis* pupated (Table 6).

Second Evaluation at 21°±1°C Slopes, standard errors of the slopes, and LC_{50}'s and LC_{90}'s with corresponding 95% fiducial limits for each chemical are shown (Table 7). No

mortality seen in control beakers with water and water plus substrate added.

Slope comparisons showed two groupings. Temephos in water alone appeared to be the most toxic. All other chemicals at all treatments had similar slopes with similar toxicities. Permethrin and dibrom in water plus substrate showed no mortality. The relative potency of these chemicals was the same as at 19±1°C.

In tests on non-targets, the *Uca* spp., and controls exhibited no mortality. Most of the larvae tested were *C. hollensis* (84%) and the remainder were *C. furens* (16%). Only 22 or 7.5% of the larvae pupated (Table 8).

Two tests at different temperatures were conducted to evaluate the toxicity of four larvicides against biting midge larvae at four different concentrations. Temephos and dibrom are organophosphates, and resmethrin and permethrin are pyrethroids. The addition of soil substrate reduced the toxicity of each of the larvicide, as reported previously for *Culicoides* (Fox et al. 1968). It is believed (Westberry & Hagan unpublished data) that since the majority of the larvae were 4th instar, the post-treatment pupation observed was simply a result of the natural life cycle. Whether any morphological or physiological changes may later result from the agents is not known.

The toxicity data from these studies support the

results obtained on the relative potency of these four larvicides. In the laboratory, Fox et al. (1968) and Kline et al. (1985) found that temephos was the most effective against field collected *C. furens* larvae. Kline also found that plastic cups and substrate reduced the toxicity of the agent. Apperson (1975) found that temephos was the most biologically effective agent against *C. variipennis*, a non-estuarine species. Temephos was also found to be effective against *C. molestus* (Skuse) in Australia (Ferguson & Cawthorne 1980).

Wall and Marganian (1971 & 1973) found, in field studies, that temephos was effective in reducing the number of larvae, without reducing the number of non-target organisms. Campbell and Denno (1976) found that temephos had little effect on the non-target organisms in the New Jersey marsh. In Australia, Kay et al. (1973) found that temephos had little effect on non-target organisms in the salt marsh. The *Uca* spp. that died was because they had been in the laboratory for a long period of time and were becoming lethargic.

All larvicides, except dibrom which had worked well in controlling adults, merit further testing in the laboratory and in the field. Temephos seems to be the most effective agent for controlling biting midge larvae in the laboratory, therefore field testing is necessary to test the true

effectiveness.

The salt marsh intertidal zone is a unique habitat and serves as a boundary between the estuarine and upland environments. As the human population density of the coastal areas increases (e.g. Glynn County population increased >150% from 1940-1980 (Bachtel 1984)), public demand for control of biting midges will increase. Prudent future conservation of these areas will depend upon a knowledge base of the biology and life cycles of the pest species. It is my hope that the information gained from this study will aid in making wiser decisions regarding these salt marsh areas.

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Table 1. Seasonal abundances of *Culicoides* spp. in Glynn County collected by New Jersey light traps, 1990.

Species	Winter	Spring	Summer	Fall
C. furens	0	1538	1988	1432
C. hollensis	98	1246	89	466
C. melleus	7	33	56	60

Probabilities obtained using 3 X 4 contingency table testing for independence. All values are significant for seasonal distribution for each of the three species (α=0.05).

Table 2. Per trap night totals for adults collected by New Jersey light traps for each species at the six light traps with the highest number of collections. Percentages are in parentheses.

Species	LT 1	LT 2	LT 4	LT 9	LT 12	LT 14
C. furens	1,115	173	499	1,161	114	242
	(22)	(3)	(10)	(23)	(8)	(5)
C. hollensis	742	154	115	198	117	115
	(39)	(8)	(6)	(10)	(6)	(6)
C. melleus	64	8	7	7	14	13
	(41)	(5)	(4)	(4)	(9)	(5)
Total	1,921	335	621	1,366	245	370
	(27)	(5)	(9)	(19)	(7)	(5)

Probabilities obtained using X^2 analysis. All totals are significant for light trap with $P \leq 0.05$.

Table 3. Range of temperature preferendia for two species of larval *Culicoides*.

Temperature	*C. furens*	*C. hollensis*
20.0-29.9°C	34	65
30.0-39.9°C	51	25
40.0-49.0°C	5	0

*Probabilities obtained using 2 X 3 contingency table testing for independence. All values are significant for temperature with both species (α=0.05).

Table 4. Abundance of *Culicoides* spp. in Glynn County, Georgia and the relationship with the phase of the moon (light and dark cycles).

Species	Month	U value	n_1, n_2
C. furens	August	10	3,4
C. furens	September	15	5,4
C. hollensis	April	12	4,5
C. hollensis	October	18^{+}	5,4
C. melleus	August	7	3,4
C. melleus	Septmeber	14.5	4,5

Probabilities obtained using $\alpha=0.05$. All three species were not siginificant for moon phase, except *C. hollensis* in October ($P \leq 0.05$).

Table 5. Laboratory efficacy (19 ± 1°C) of four larvicides against field-collected *Culicoides* larvae from a St. Simons, Georgia salt marsh.

Chemical	No. tests[1]	Treatment	LC_{50} (ppb)	95% Fiducial Limits	LC_{90} (ppb)	95% Fiducial Limits	Slope[2]	SE
temephos	2	water	1.59	0.08-1.91	2.29	1.97-3.61	1.84a	0.02
	2	water/soil	2.38	2.16-3.07	2.86	2.56-6.20	2.36a	0.03
resmethrin	2	water	1.71	1.21-1.95	2.16	1.92-2.83	2.88a	0.03
	2	water/soil	2.15	1.80-2.33	2.51	2.33-3.05	3.63a	0.04
permethrin	2	water	1.84	1.40-2.07	2.33	2.10-2.86	2.61a	0.02
	2	water/soil	2.06	1.74-2.23	2.40	2.23-2.83	3.88a	0.04
dibrom	2	water	2.90	∞[3]	3.33	∞	2.97a	0.11
	2	water/soil	5.04	∞	7.42	∞	0.54a	0.03

[1]Each test consisted of at least four discriminating concentrations of insecticide; duplicate beakers containing 4 larvae each were used at each concentration.
[2]Numbers followed by the same letter do not differ significantly at $P \leq 0.05$.
[3]∞ = infinite

Table 6. Identification and number of larvae in each larvicide at 19±1°C (F=*C. furens*, H=*C. hollensis*, M=*C. melleus*).

Chemical	[C] PPB	Treatment	n	Species
temephos	1000	water	8	8 H
		water/soil	8	8 H
	4000	water	6	6 H
		water/soil	9	9 H
	7000	water	7	7 H
		water/soil	9	9 H
	10,000	water	7	7 H
		water/soil	7	7 H
resmethrin	1000	water	8	7 H, 1 F
		water/soil	8	8 H
	4000	water	8	8 H
		water/soil	6	5 H, 1 F
	7000	water	7	7 H
		water/soil	6	6 H
	10,000	water	6	5 H, 1 F
		water/soil	8	7 H, 1 F
permethrin	1000	water	6	6 H
		water/soil	9	8 H, 1 F
	4000	water	8	8 H
		water/soil	7	6 H, 1 F
	7000	water	9	9 H
		water/soil	6	6 H
	10,000	water	8	7 H, 1 F
		water/soil	8	8 H
dibrom	1000	water	7	7 H
		water/soil	8	8 H
	4000	water	6	6 H
		water/soil	8	8 H
	7000	water	8	7 H, 1 F
		water/soil	8	8 H
	10,000	water	7	7 H
		water/soil	8	8 H

Table 7. Laboratory efficacy (21 ± 1°C) of four larvicides against field-collected *Culicoides* larvae from a St. Simons, Georgia salt marsh.

Chemical	No. tests[1]	Treatment	LC_{50} (ppb)	95% Fiducial Limits	LC_{90} (ppb)	95% Fiducial Limits	Slope[2]	SE
temephos	2	water	1.60	∞[3]	2.54	∞	1.37a	0.04
	2	water/soil	2.79	∞	3.18	∞	3.30b	0.09
resmethrin	2	water	2.35	2.21-2.47	2.59	2.47-3.16	5.24b	0.06
	2	water/soil	2.15	1.80-2.33	2.51	2.33-3.05	6.31b	0.18
permethrin	2	water	2.37	2.12-2.61	2.73	2.53-4.09	3.52b	0.04
	2	water/soil	0.00		0.00			
dibrom	2	water	2.97	∞	3.33	∞	2.80b	0.08
	2	water/soil	0.00		0.00			

[1]Each test consisted of at least four discriminating concentrations of insecticide; duplicate beakers containing 4 larvae each were used at each concentration.
[2]Numbers followed by the same letter do not differ significantly at $P \leq 0.05$.
[3]∞ = infinite

Table 8. Identification and number of larvae in each larvicide at 21±1°C (F=*C. furens*, H=*C. hollensis*, M=*C. melleus*).

Chemical	[C] PPB	Treatment	n	Species
temephos	1000	water	8	8 H
		water/soil	9	7 H, 1 F
	4000	water	10	10 H
		water/soil	8	10 H, 3 F
	7000	water	8	8 H
		water/soil	9	7 H, 2 F
	10,000	water	8	8 H
		water/soil	7	7 H
resmethrin	1000	water	8	7 H
		water/soil	9	7 H, 2 F
	4000	water	7	5 H
		water/soil	6	6 H
	7000	water	10	10 H
		water/soil	10	7 H, 3 F
	10,000	water	8	8 H
		water/soil	8	5 H, 3 F
permethrin	1000	water	8	7 H, 1 F
		water/soil	4	3 H, 1 F
	4000	water	9	8 H, 1 F
		water/soil	7	6 H, 1 F
	7000	water	6	6 H
		water/soil	9	6 H, 3 F
	10,000	water	8	7 H, 1 F
		water/soil	7	1 H, 6 F
dibrom	1000	water	8	7 H, 1 F
		water/soil	8	6 H, 2 F
	4000	water	8	7 H, 1 F
		water/soil	8	8 H
	7000	water	10	7 H, 1 F
		water/soil	6	2 H, 4 F
	10,000	water	9	9 H
		water/soil	7	3 H, 4 F

Fig. 1. Research area in Glynn County, Georgia with Sea Island and St. Simons Island, Georgia. The two larval sampling sites were Site 1, North Sea Island and Site 2, North St. Simons Island.

U. S. DEPARTMENT OF AGRICULTURE
SOIL CONSERVATION SERVICE
UNIVERSITY OF GEORGIA
COLLEGE OF AGRICULTURE
RGIA AGRICULTURAL EXPERIMENT STATION

GENERAL SOIL MAP

GLYNN COUNTY, GEORGIA

Scale 1:253,440

1 0 1 2 3 4 Miles

1 0 4 8 Km

Fig. 2. Three major elevation and vegetation zones in the salt marsh study site on north Sea Island, Ga. The predominant vegetation is listed under each zone. MHW, mean high water; MSL, mean sea level; MLW, mean low water (Magnon et al. 1990).

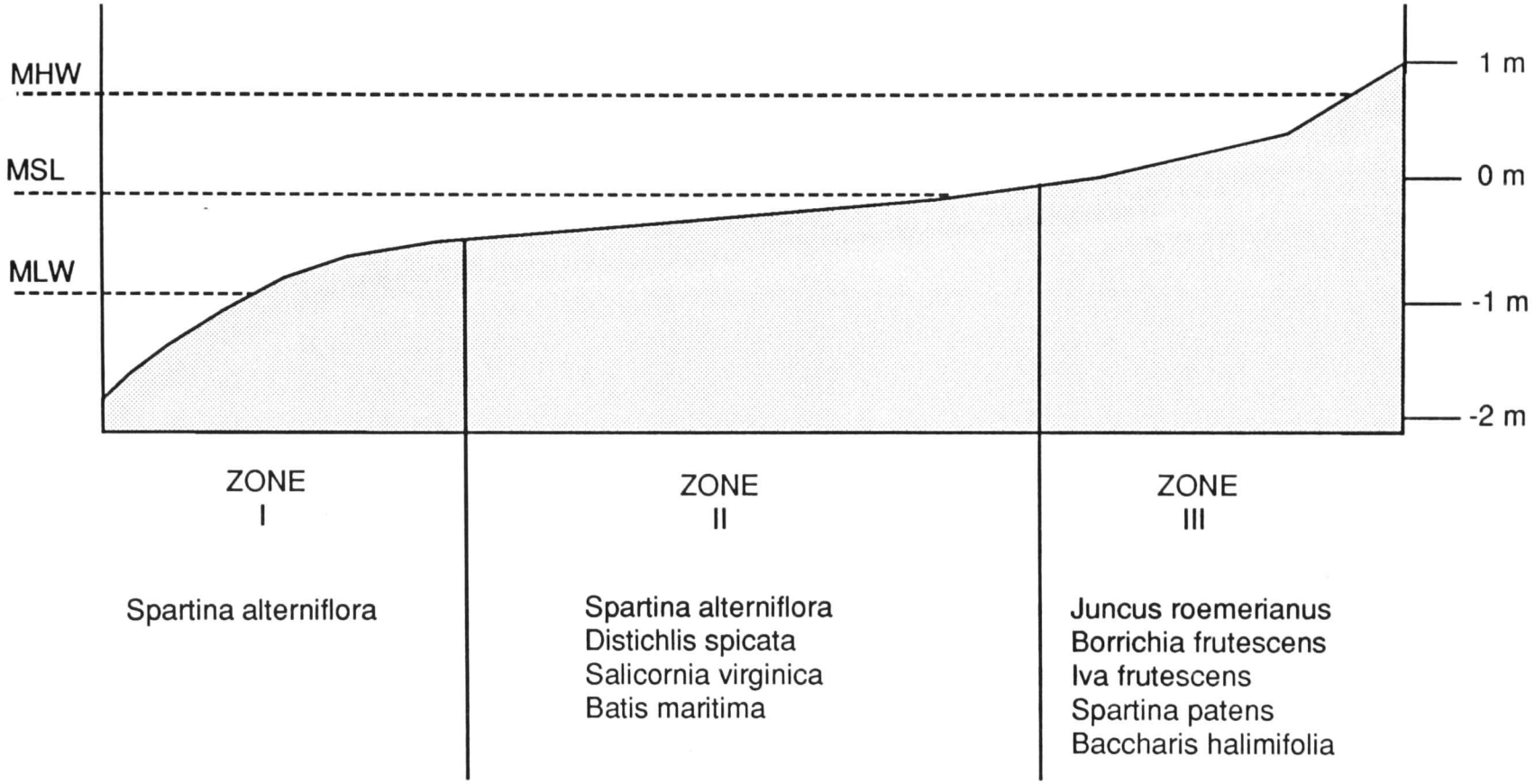

MHW
MSL
MLW
1 m
0 m
-1 m
-2 m
ZONE
I
ZONE
II
ZONE
III
Spartina alterniflora
Spartina alterniflora
Distichlis spicata
Salicornia virginica
Batis maritima
Juncus roemerianus
Borrichia frutescens
Iva frutescens
Spartina patens
Baccharis halimifolia

Fig. 3. Three major elevation and vegetation zones in the salt marsh study site on north St. Simons Island, Ga. The predominant vegetation is listed under each zone. MHW, mea high water; MSL, mean sea level; MLW, mean low water.

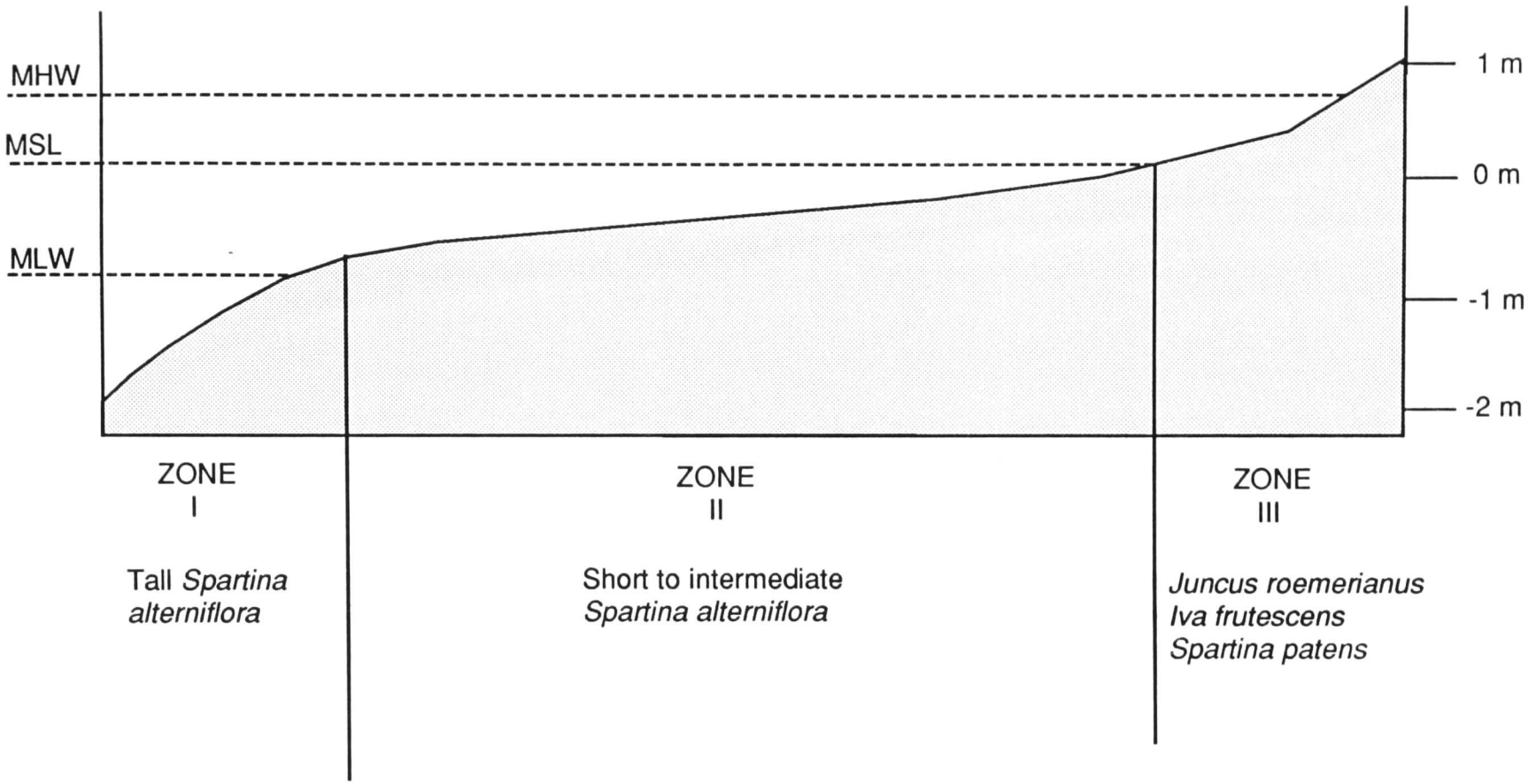

MHW
MSL
MLW
1 m
0 m
-1 m
-2 m
ZONE
I
ZONE
II
ZONE
III
Tall *Spartina alterniflora*
Short to intermediate *Spartina alterniflora*
Juncus roemerianus
Iva frutescens
Spartina patens

Fig. 4. Glynn County map showing the locations of the 15 New Jersey light traps.

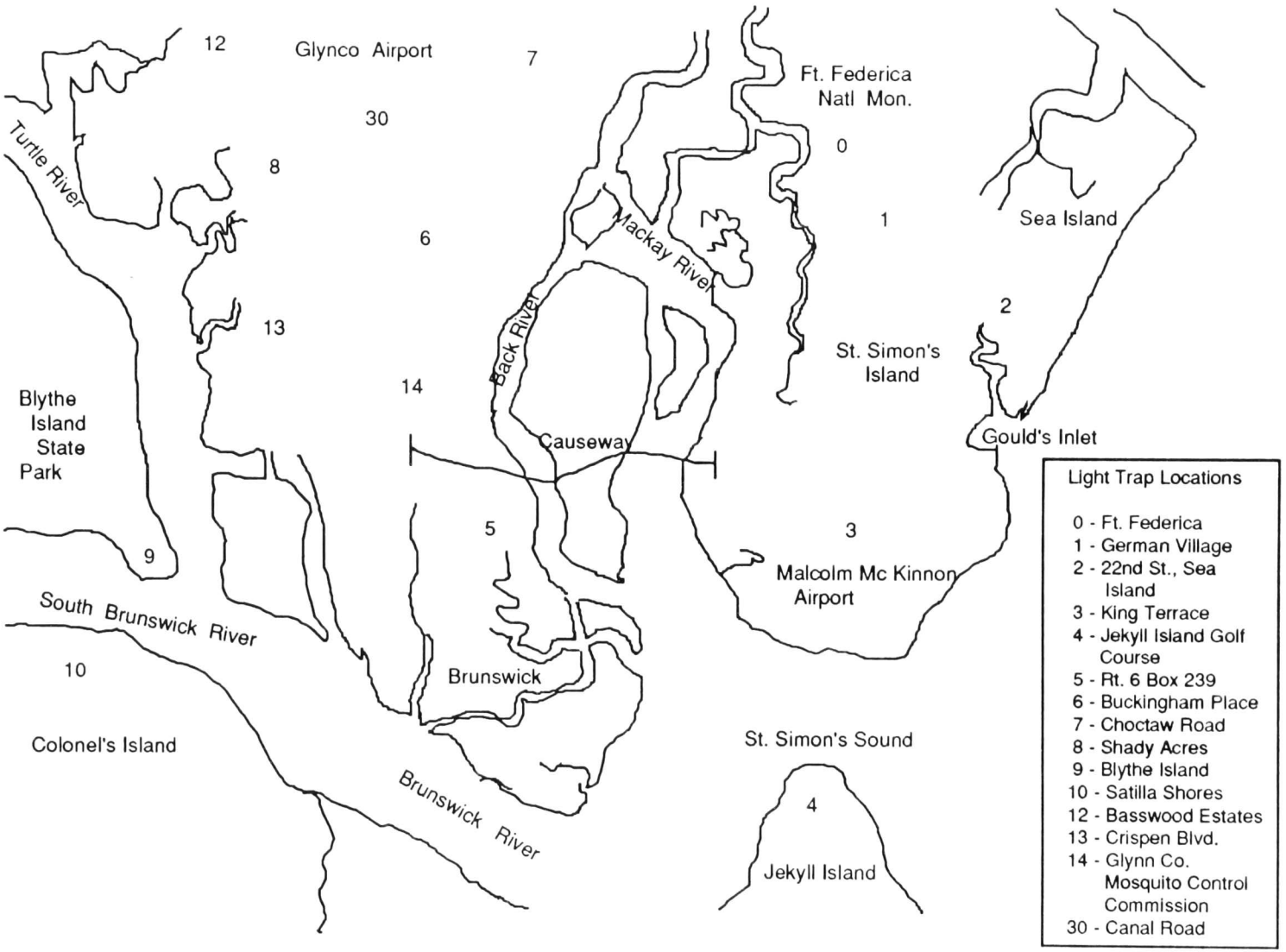

12
Glynco Airport
7
30
Ft. Federica
Natl Mon.
0
Turtle River
8
1
Sea Island
6
Mackay River
Back River
2
13
St. Simon's
Island
14
Blythe
Island
State
Park
Causeway
Gould's Inlet
5
3
9
Malcolm Mc Kinnon
Airport
South Brunswick River
10
Brunswick
Colonel's Island
St. Simon's Sound
Brunswick River
4
Jekyll Island
Light Trap Locations
0 - Ft. Federica
1 - German Village
2 - 22nd St., Sea Island
3 - King Terrace
4 - Jekyll Island Golf Course
5 - Rt. 6 Box 239
6 - Buckingham Place
7 - Choctaw Road
8 - Shady Acres
9 - Blythe Island
10 - Satilla Shores
12 - Basswood Estates
13 - Crispen Blvd.
14 - Glynn Co. Mosquito Control Commission
30 - Canal Road

Fig. 5. Apparatus used to test temperature preferendia.

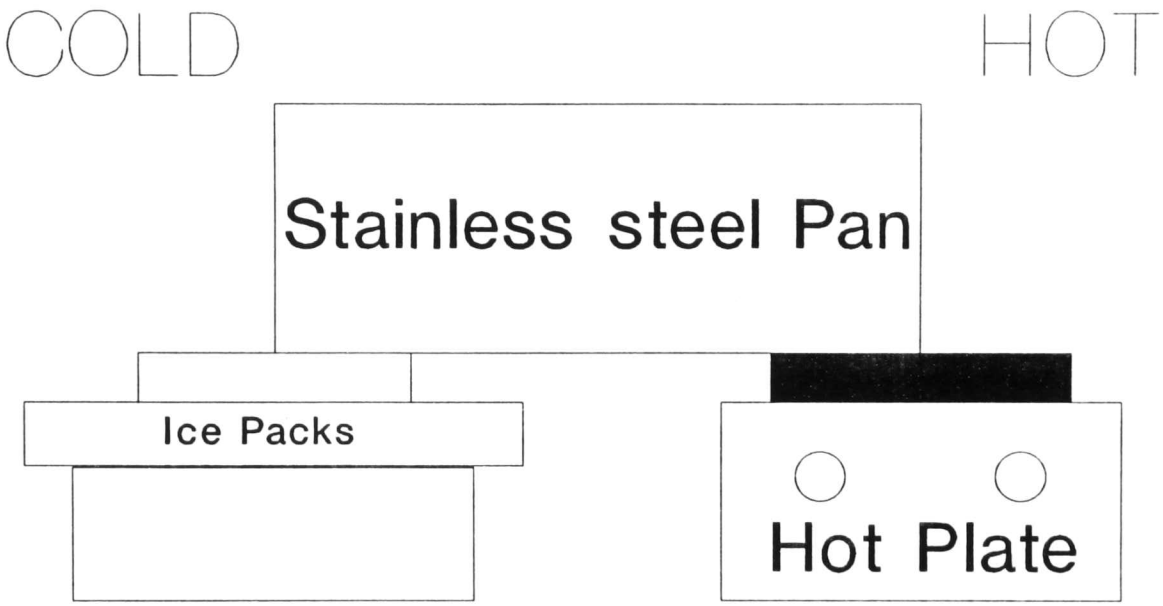

COLD
HOT
Stainless steel Pan
Ice Packs
Hot Plate

Fig. 6. Monthly incidence of adult *C. furens* as monitored by the three most productive light traps (LT) during April through December 1990.

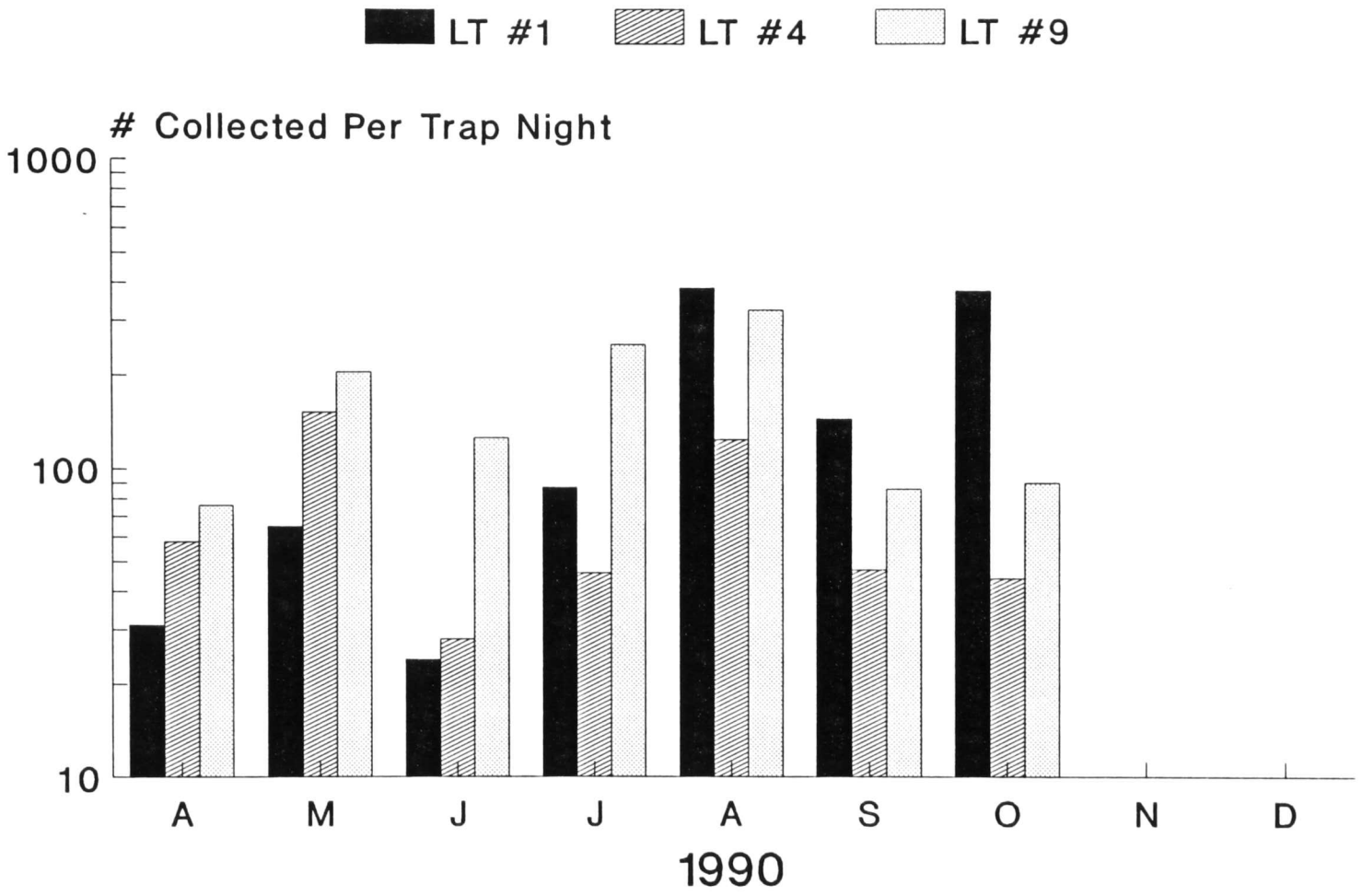

LT #1
LT #4
LT #9
Collected Per Trap Night
1000
100
10
A
M
J
J
A
S
O
N
D
1990

Fig. 7. Monthly incidence of adult *C. hollensis* as monitored by the three most productive light traps during April through December 1990.

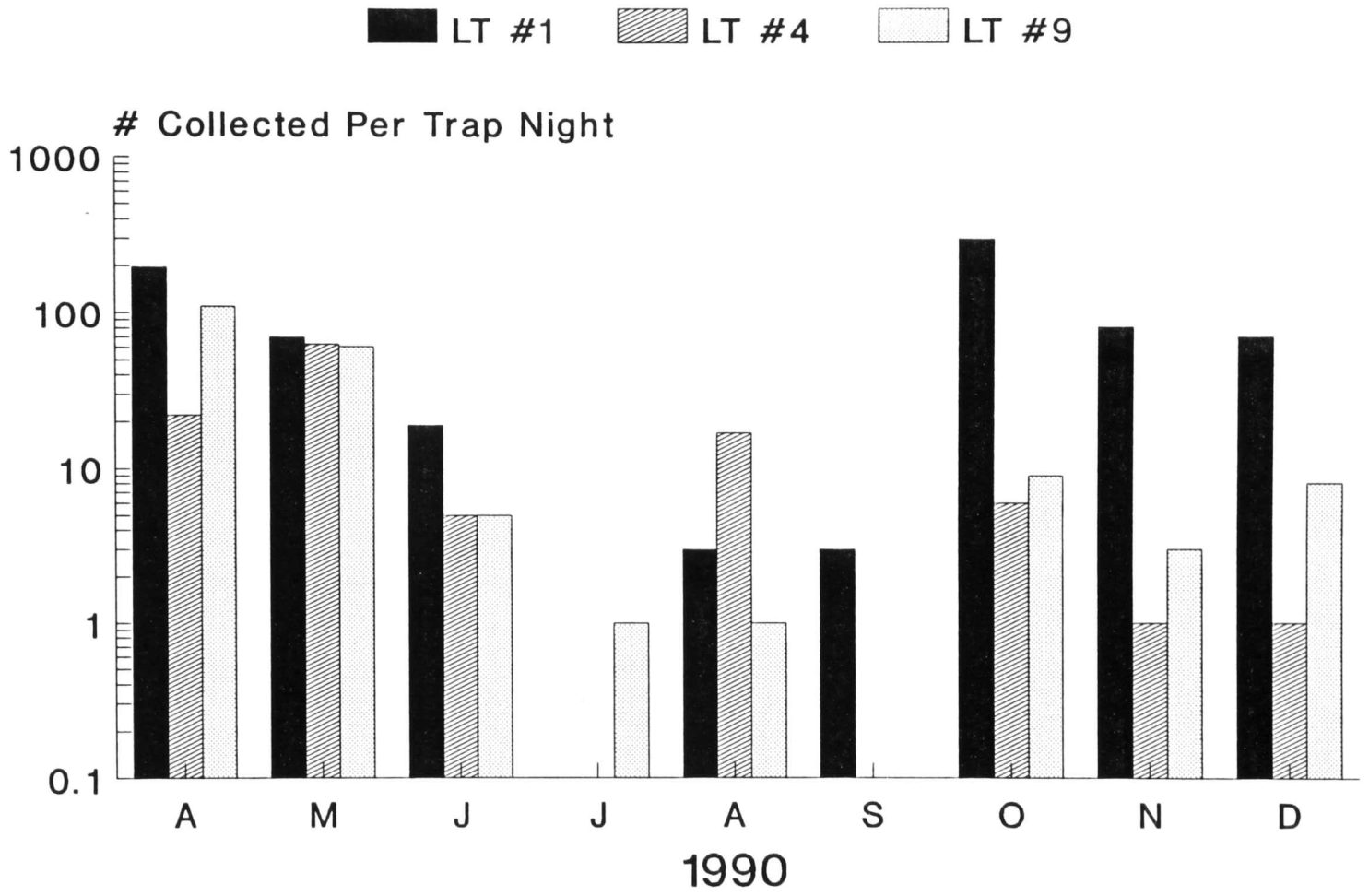

LT #1
LT #4
LT #9
Collected Per Trap Night
1000
100
10
1
0.1
A
M
J
J
A
S
O
N
D
1990

Fig. 8. Monthly incidence of adult *C. melleus* as monitored by the three most productive light traps during April through December 1990.

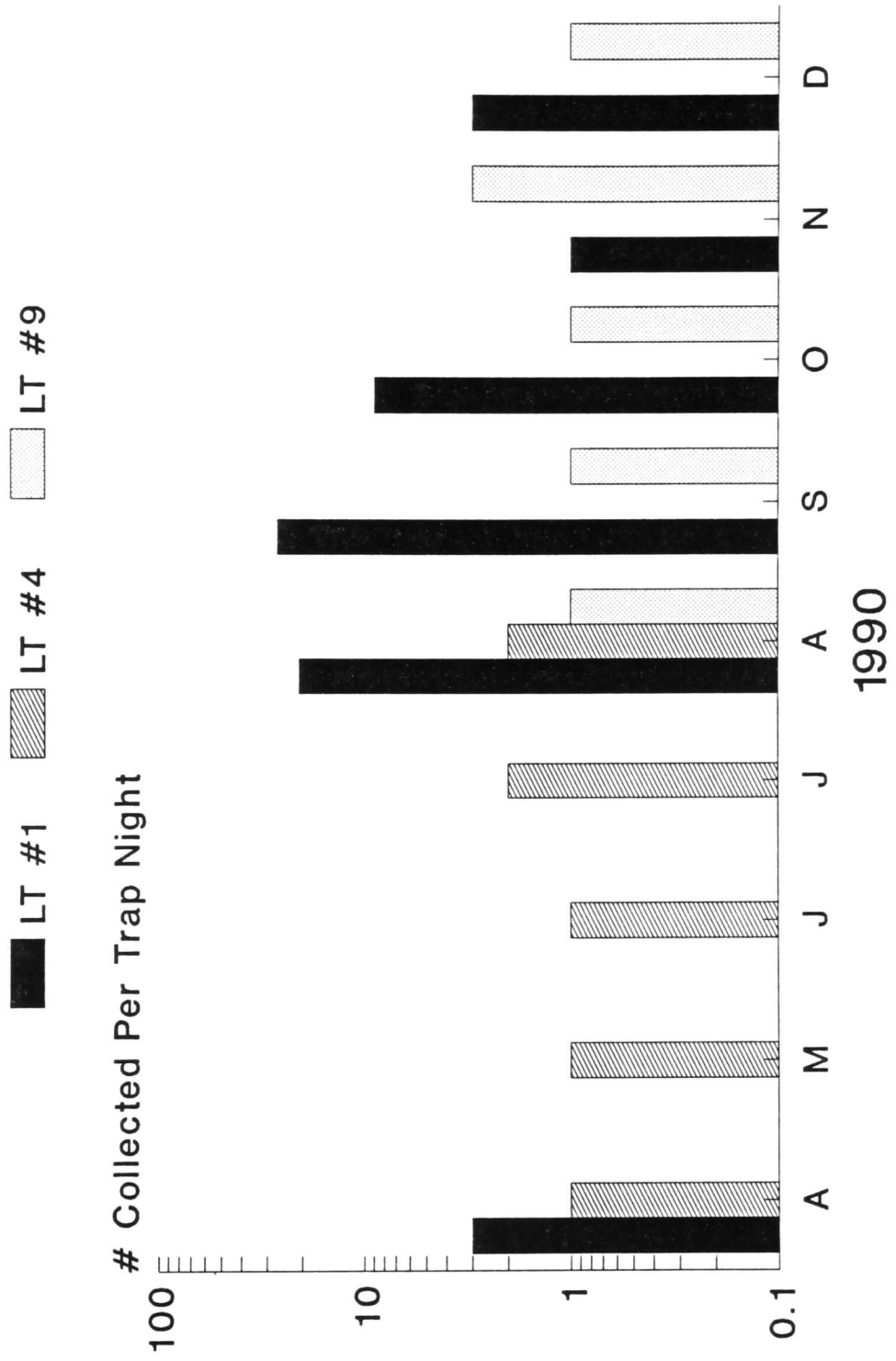

LT #1
LT #4
LT #9
Collected Per Trap Night
100
10
1
0.1
A
M
J
J
A
S
O
N
D
1990

Fig. 9. Number of larval *Culicoides* spp. collected from February 1990 through April 1991. From October through December 1990 larvae were collected using hand trowels instead of post-hole diggers.

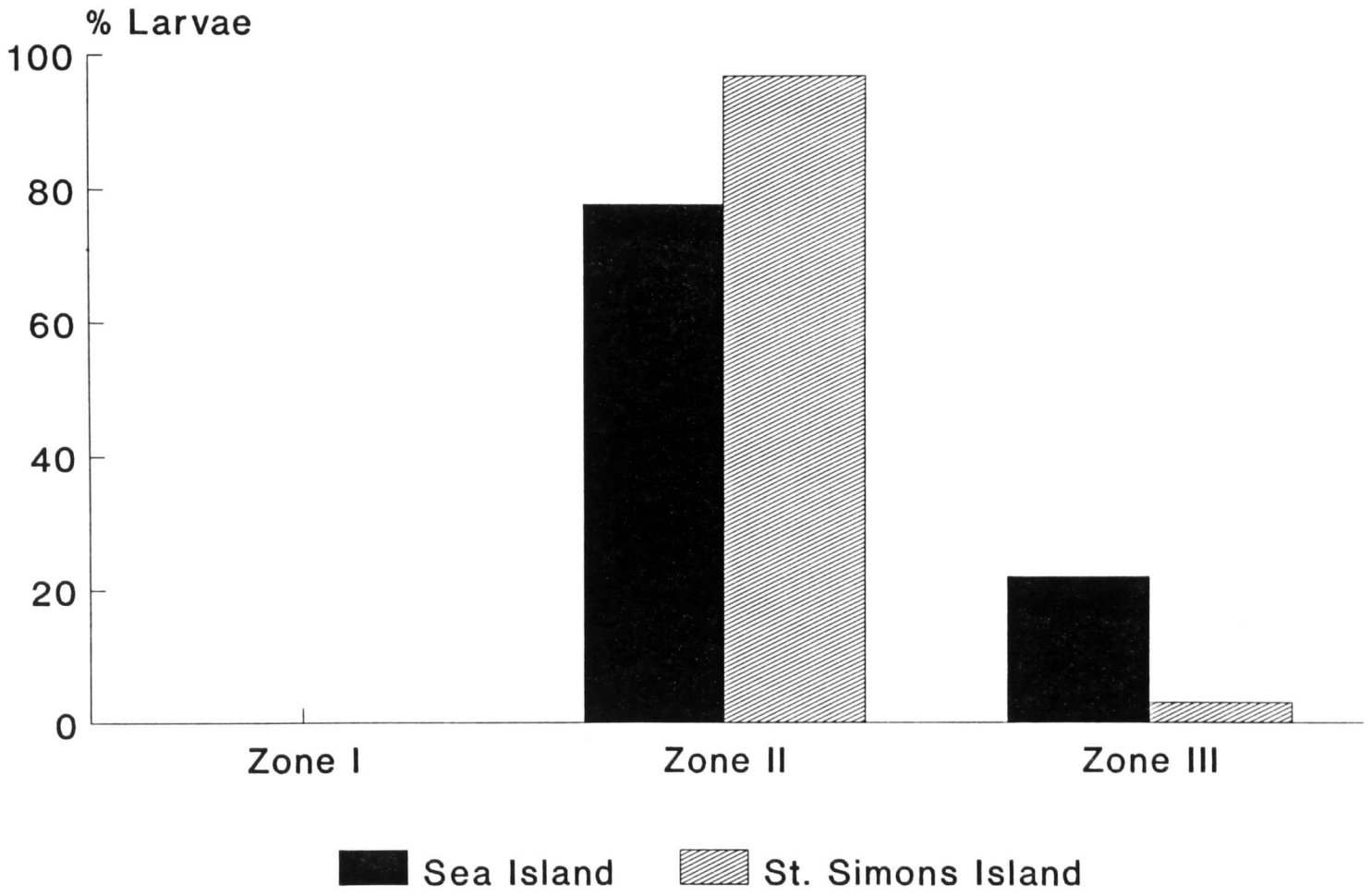

% Larvae
100
80
60
40
20
0
Zone I
Zone II
Zone III
Sea Island
St. Simons Island

Fig. 10. Distribution of larval *Culicoides* spp. in thr elevation zones at the two different research sites.

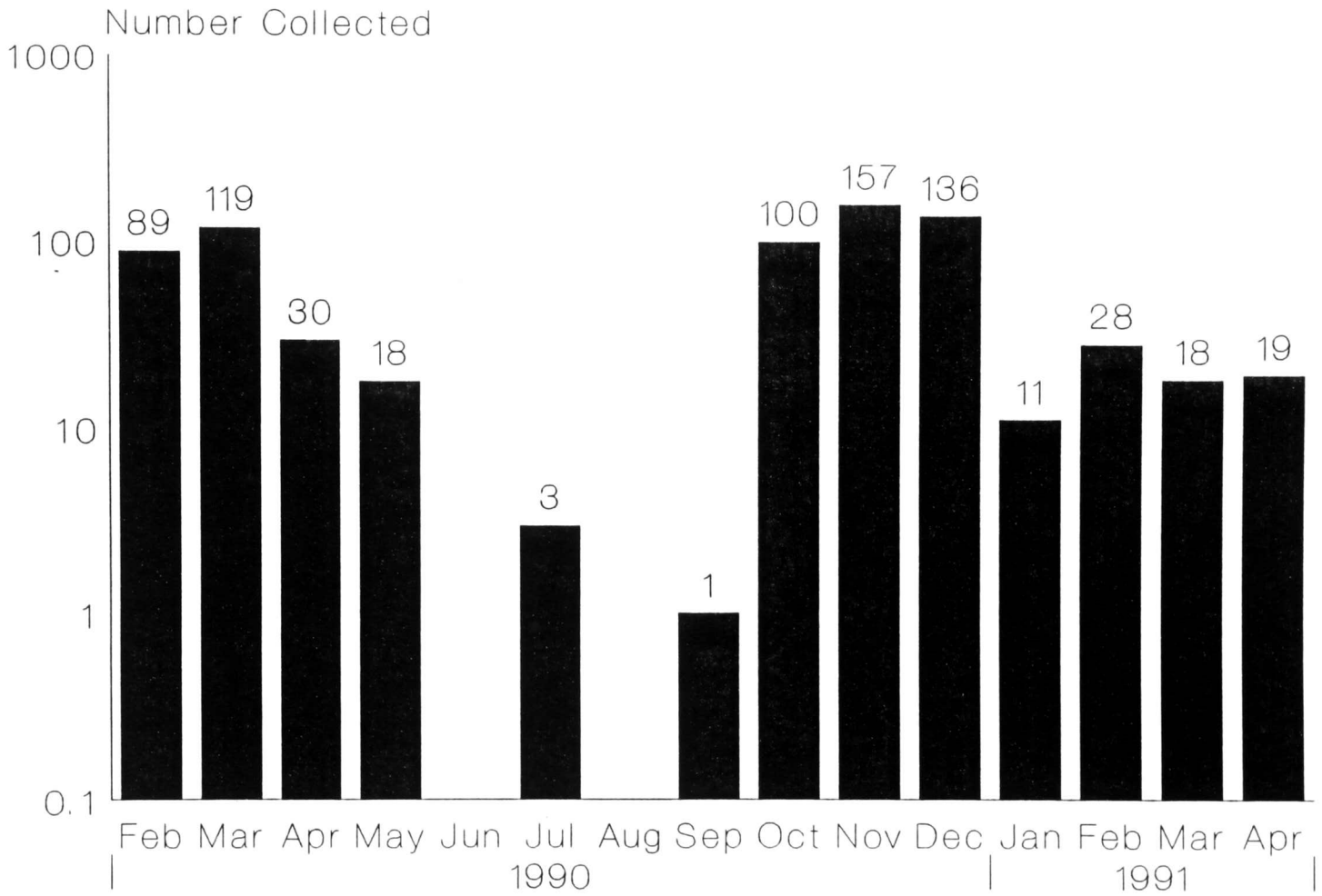

Number Collected
1000
100
10
1
0.1
89
119
30
18
3
1
100
157
136
11
28
18
19
Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec Jan Feb Mar Apr
1990
1991

Fig. 11. Frequency distribution of larval *C. furens* at various temperature increments.

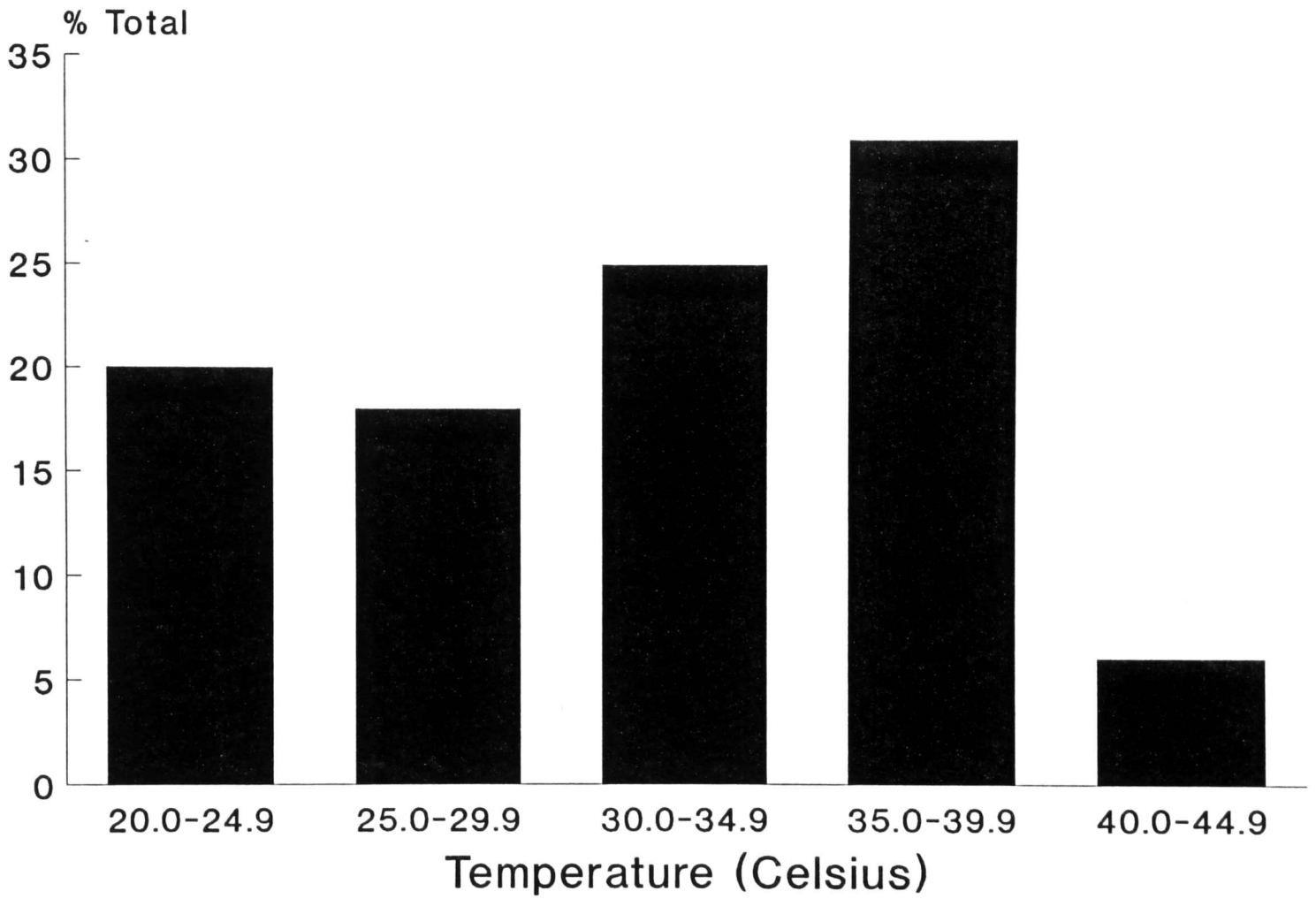
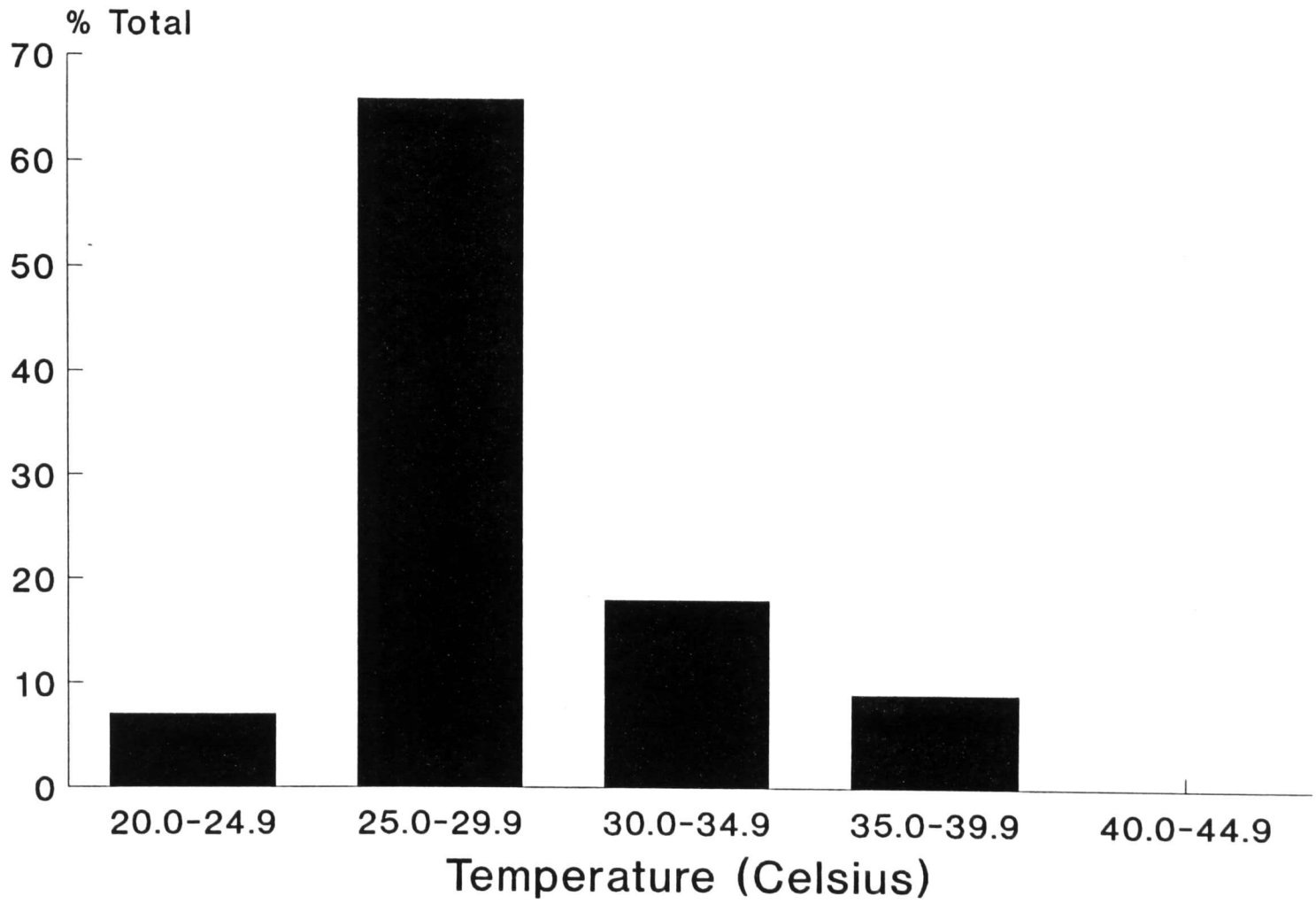

% Total
35
30
25
20
15
10
5
0
20.0-24.9
25.0-29.9
30.0-34.9
35.0-39.9
40.0-44.9
Temperature (Celsius)

Fig. 12. Frequency distribution of larval *C. hollensis* various temperature increments.


% Total
70
60
50
40
30
20
10
0
20.0-24.9
25.0-29.9
30.0-34.9
35.0-39.9
40.0-44.9
Temperature (Celsius)